Investigation of the effect of substrate and electrode modifications on the electrical output of Microbial Fuel Cell

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1-38

Abstract

The world is currently being stripped of its natural resources, to produce electricity with fossil fuels, polluting it further. Microbial Fuel Cells (MFCs) are a viable alternative energy source less damaging to the environment that use electrogenic bacteria. This project aims to investigate whether the common gut bacterium *Lactobacillus acidophilus (L.A.)* can generate electricity, and which substrate and electrode modifications yield the highest electrical output. It is hypothesised that ethanol and modified electrodes will yield the highest electrical output. First, an MFC was constructed. Different substrates of equal volume were used with equal concentration of bacteria (0.7 abs with 660 nm wavelength) inside the anode chamber of MFC to investigate the effect of substrate modification on voltage of MFC. A data logger was used to measure voltage. All other factors were kept constant. The MFC with ethanol was found to have the highest voltage of 0.35mV. Second, different electrodes were used, and polarisation curves were plotted to investigate the effect of electrode modification on electrical output of MFC. This was done by varying resistance of MFC and measuring voltage across the anode and cathode for 4 different sets of electrodes. All other factors were kept constant. The MFC with Sulfuric acid-treated carbon rod was found to have the highest power density at 0.527mW / cm^2. However, chemical modification increased resistance of the treated carbon rods. *L.A.* generated voltage of 0.35mV using ethanol as substrate and plain carbon rod as electrode. This project has made it more economically viable to use MFC for sustainable electrical production on a large scale as *L.A.* is more common and easier to culture than other bacteria such as *Geobacter Sulfurruducens*, the most promising electrogenic bacteria. The substrates and electrodes used were also relatively inexpensive.

1. Introduction

Exhaustion of fossil fuel and climate change has increased the demand for renewable sources of energy. Microbial Fuel Cell (MFC) has been gaining popularity as a renewable source of energy that can use electrogenic microbes to break down and recycle organic substrates to produce electricity. The electricity produced is thus clean and safe which establishes this technology as less harmful to the environment (Nitisoravut, Thanh & Regmi, 2017).

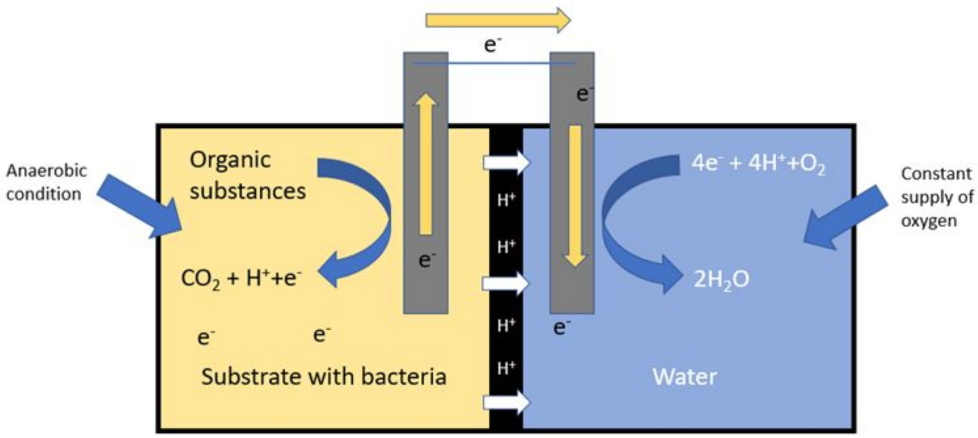

Figure 1: Schematic diagram of microbial fuel cell

MFC consists of an anode, a cathode, an electrolyte medium which are connected using electrodes, the Proton Exchange Membrane (PEM) and microorganisms as shown in Figure 1. Microorganisms and electrodes are the main components of an MFC that could significantly affect its cost and performance (Cloudhurry *et al.*, 2017).

Exotic microorganisms such as *Geobacter sulfurreducens* are often used in MFCs due to their relatively high rate of electrical output (Bond & Lovely, 2003; Rotaru *et al*., 2018 ; Raebey &Verstraete, 2005). In a research study into microbial fuel cells running on various types of bacteria, *Geobacter Sulfurreducens* was found to generate a current of 0.4mA running on Acetate with plain carbon electrodes. However, recent research has shown that there are many other species of electrogenic bacteria which can be possibly used in MFCs.

Lactobacillus, a genus of gut bacteria, is commonly found in yoghurts and probiotic drinks. A bacteria in the genus, *Lactobacillus Bulgaricus* was found to be able to generate around 0.1 mA/h when ran for 3 hours (Arbianti *et al*., 2013). *L.A.* is a gram-positive bacteria that functions best at low pH values of below 5.0 (Hood & Zoitola, 1988), and a temperature of

37.0°C (Wheater, 1955). There is promising potential for *L.A.* as a bacteria to be used in MFCs. This is because although bacteria of the Lactobacillus genus have shown promising results, no research has been done specifically about *L.A*.

In the last decade, many studies have also been performed to investigate the factors influencing the performance of MFCs. Surface modification of anode using surfactant has great influence on the electrical performance of a MFC. (Song *et al.*, 2015). Compared to other modification techniques, surface modification is also one of the fastest and most convenient methods for modifying electrode materials. Moreover, electrochemical modification can be performed with low-cost chemical compounds and is overall cost-effective. (Wang *et al.*, 2009).

Substrate is another important factor in the performance of MFCs. The major substrates that have been experimented include various kinds of artificial and real wastewaters and lignocellulosic biomass. The current and power yields are relatively low at present but it is expected that the amount of electric current these systems produce will increase greatly when technology and knowledge about these unique systems improve, providing a sustainable way of directly converting lignocellulosic biomass or wastewaters to useful energy (Pant *et al*., 2009).

2. Objectives and Hypotheses

2.1 Objectives

1. Investigate the ability of *L.A.* to act as a catalyst for obtaining electrical energy from simple substrates.
2. Investigate which substrate is optimal for production of energy.
3. Investigate which electrode modification will result in optimal electrical output of MFC.

2.2 Hypotheses

1. *L.A.* can be used in MFCs to generate current.
2. Most current is generated when Ethanol substrate is used.

3. Modified electrodes have higher power densities and lower resistances than control electrodes.

3. Methodology

3.1. Construction of fuel cell

A microbial fuel cell was constructed with silicon glue, hot glue, acrylic boards and PEM which was purchased from Fuel Cell Store (College Station, Texas, USA). (Refer to appendix A, B and C for the set-up of the MFC)

3.2. Preparation of Substrates and buffer solutions

Glucose substrate (1M), sucrose substrate (1M), methyl propionate substrate (1M), methyl butyrate substrate (1M), acetate substrate (1M) and ethanol substrate (10mM) were prepared. Ethanol substrate was lower in concentration as it acts as a disinfectant at higher concentration which could kill the bacteria. Buffer solution was used to maintain the pH of the substrates throughout the experiment. Solution A was made by dissolving 10.5g of citric acid in 500 mL of deionised (DI) water and solution B was made by dissolving 14.7 g of trisodium citrate into another 500 mL of DI water. These 2 solutions were then mixed in a 41:59 ratio to form a buffer solution of pH 5, which is the optimum pH for *L.A.*

3.3. Inoculation of bacteria

L.A. were inoculated into 5 test tubes of 15 mL of MRS broth. The cultures were then incubated at 35°C overnight. The cultures were centrifuged to collect the bacteria. The bacteria was then redispersed in DI water and centrifuged again for 2 times. UV-vis-spectroscopy was used to ensure consistency in bacteria concentration at 0.7 absorbance at 660 nm wavelength and the concentrations were adjusted using serial dilution.

3.4. Modification of electrodes

Three different methods were used to treat the carbon rods. The first batch of carbon rods were treated with concentrated sulfuric acid (6M) and then heated at 250 °C for 2 hours. The second batch of carbon rods were soaked in Nitric acid for 5 hours and then cleaned with DI water until neutral pH. The third batch of carbon rods were soaked in ammonium peroxydisulfate and concentrated sulfuric acid for 15 minutes. The carbon rods treated with the 3 methods stated above were exposed in the microwave for 10s, washed under water until neutral pH and dried in open air to obtain the oxidised carbon rods. The carbon rods were mixed with anionic surfactants (sodium dodecyl sulfate and polytetrafluoroethylene, 20mM). The mixture was subjected to microwave radiation for 20 minutes. The final treated CRs were dried.

3.5. Collection and analysis of data

3.5.1. Overnight data logging

Overnight data logging was done using a data logger to investigate the effect of substrate modifications on the output voltage of MFC. (Refer to appendix D for set-up of overnight data logging.)

3.5.2. Polarisation Test

As seen in Figure 2, a schematic diagram of the set up for polarisation test, a variable resistor was connected in series with MFC and a voltmeter was connected in parallel to measure the voltage. Stable voltages were recorded down as the external resistance was manually changed from 100k Ω to 0 Ω. Current (mA) was measured by dividing voltage (measured by multimeter) with external resistance ($I = \frac{V}{R_{ext}}$). Current density (mA/cm^2) was calculated by dividing the current with the cross-sectional area of the electrode ($J = \frac{I}{\pi r^2}$). The power density (mW/cm^2) was calculated by dividing the product of current and voltage with the cross sectional area of the electrode ($Power\ density = \frac{VI}{\pi r^2}$). Graphs of power density against current density were plotted to find the maximum power density. (Refer to appendix E and F for set-up of polarisation test.)

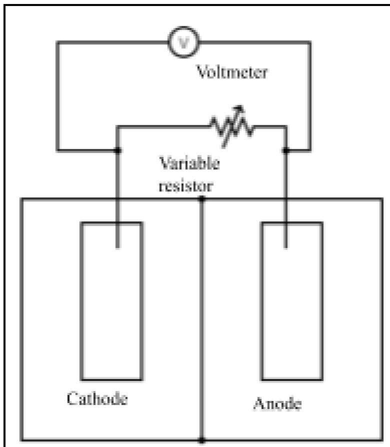

Figure 2: Set-up of polarisation test

Graphs of voltage against current density were plotted to study the different factors affecting loss in potential, namely: activation loss, ohmic loss, and concentration loss. Activation loss was caused by the slowness of the reactions taking place on the electrode surface. Ohmic loss is caused by resistance to the flow of ions in the electrolyte and electrons through the cell hardware. Concentration loss is caused by the decrease in reactant concentration at the surface of the electrodes as fuel is used. The data collected was fitted into the following equation using non linear regression:

$$V = V_{oc} - (a + b \times \ln I) - I \times R_{ohm} - c\frac{I_L}{I_L - I}$$ (Song *et al.,* 2015)

V_{oc} stands for the open circuit voltage, which was measured by disconnecting the electrical circuit when the voltage is stable. a and b are the activation loss constants. c is the concentration loss constant. I_L is the limiting current, which refers to the maximum current that could be used to get a desired electrode reaction without undue interference, such as that may come from polarisation. Based on graphs plotted on Mathematica with arbitrary values, higher a and b values indicated higher effects of activation loss. Thus, the a and b values of the polarisation curves were used as a criteria when comparing the different electrodes.The following equations were used as approximations of the activation resistance and concentration resistance:

$$R_{act} = \frac{a + b \times \ln(I)}{I}$$ (Estimation of activation resistance, Song *et al*. 2015)

$$R_{conc} = \frac{[c \times \ln \frac{I_L}{I_L - I}]}{I}$$ (Estimation of concentration resistance, Song *et al.* 2015)

4. Results and discussion

4.1. Data logging

Figure 3 shows that ethanol was the most effective substrate, as it generated the greatest voltage at about 0.35 V. This was followed by sucrose and glucose, with a voltage of 0.25 V and 0.11 V respectively. Meanwhile, our control setup without bacteria produced a voltage of 0.03 V, which was the background voltage. Voltage of MFC with propionate as substrate had a sharp decrease in voltage in the first 30 minutes as anode solution leaked around the membrane and mixed with a cathode solution. Figure 4 shows that MFCs reached stable voltage from 17 hours onwards.

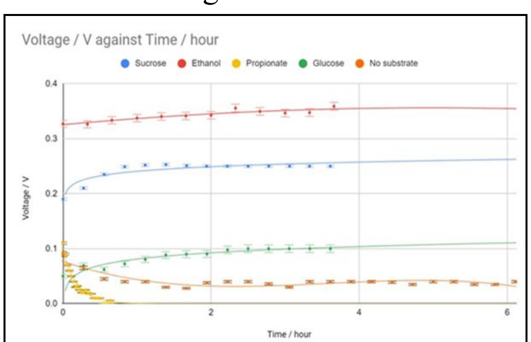

Figure 3: Graph of voltage against time for different MFCs with different substrates in the first 3-6 hours

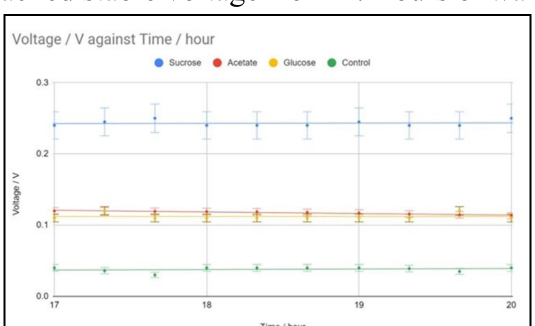

Figure 4: Graph of voltage against time for different MFCs with different substrates after 17 hours

4.2. Polarisation Test

In Figure 5, the polarisation curve of the control electrode had a broader base. The maximum point of the best fit curve of control electrode was the lowest as compared to chemically treated carbon rods. Best fit line of sulfuric acid-treated carbon rod had the highest maximum point than the remaining two, which looked very similar.

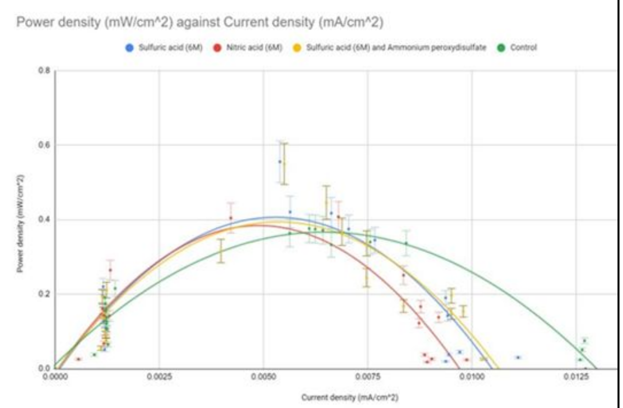

Figure 5: Graph of power density against current density for different electrodes

In Figure 6, the sharp decrease in voltage at low current density was caused by activation loss. At moderate current density, the potential decreased linearly with current due to ohmic losses. At high current density, potential drop departed from the linear relationship with current density due to concentration polarisation, caused by the decrease in reactant concentration at the surface of the electrodes as fuel was used. As seen in the voltage against current density polarisation curve, the control electrode had a broader base, while the other 3 polarisation curves were hard to distinguish visually and further analysis was needed.

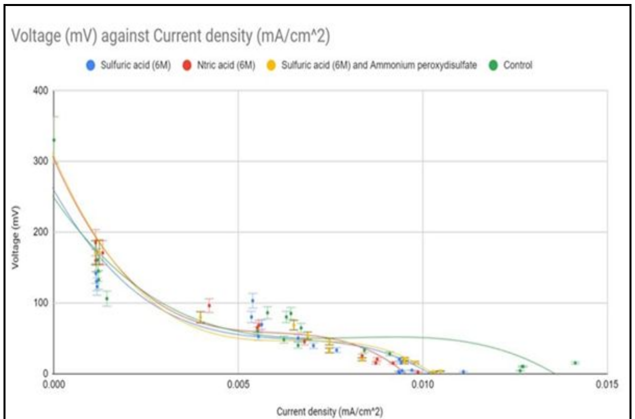

Figure 6: Graph of voltage against current density for different electrodes

As seen from Table 1, all modified electrodes had a higher maximum power density than the control electrode, with Sulfuric acid-treated electrodes having the highest power density (0.527mW).

Treatment	Maximum Power /mW(best fit line)	Maximum Power / mW (data points)
Control	0.370	0.372
H_2SO_4	0.383	0.527
H_2SO_4 + $(NH_4)_2S_2O_8$	0.383	0.522
HNO_3	0.392	0.387

Table 1: Table showing effect of treatment of carbon rod on maximum power densities,

4.3. Activation, resistance and concentration losses

Internal resistance of MFCs consists of activation resistance, ohmic resistance and concentration resistance.

Figure 7 indicates that the activation resistance was high at open circuit, and then it exponentially decreased with increase in current or decrease of the potential difference between cathode and anode. Sulfuric acid-treated carbon rods (6M) had the highest activation resistance at 185000 Ω and the resistance decreases exponentially together with the increase in current. The control electrode had the lowest activation resistance at high current, with sulfuric acid-treated carbon rods having the second least resistance. The decrease in activation resistance with the increase in current was due to the shift of anode potential to a positive direction (Song *et al.*, 2015). The order of activation resistance at maximum power density was nitric acid-treated carbon rods (4860 Ω) > sulfuric acid-treated carbon rods (639 Ω) > carbon rod treated with sulfuric acid and ammonium peroxydisulfate (545 Ω) > control (48.1Ω).

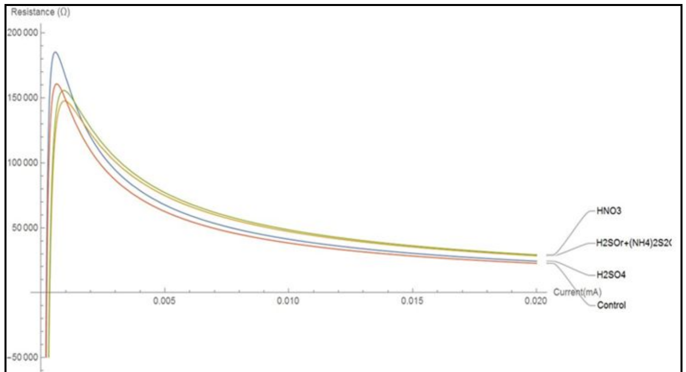

Figure 7: Graph of activation resistance against current for different electrodes

Figure 8 indicates that ohmic resistance was constant. Carbon rod treated with sulfuric acid and ammonium peroxydisulfate had the highest ohmic resistance at 28000 Ω while the control carbon rod had the lowest ohmic resistance at 9500 Ω.

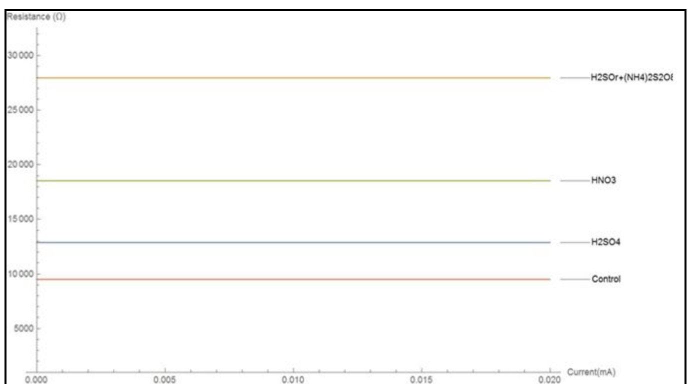

Figure 8 : Graph of ohmic resistance against current for different electrodes

Figure 9 indicates that concentration resistance increased slowly with the increase in current, but abruptly increased as it approximated limiting current. Control electrode had the highest limiting current of 0.0316 mA. The limiting currents for carbon rods treated with sulfuric acid, sulfuric acid with ammonium peroxydisulfate and nitric acid were 0.00969 mA, 0.00970 mA and 0.00906 mA respectively.

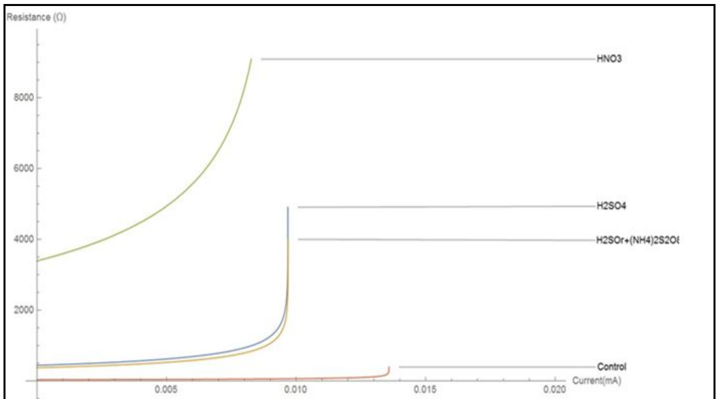

Figure 9 : Graph of concentration resistance against Current

Figure 10 indicates total internal resistance at maximum power density was mainly affected by the activation resistance as seen from the graph. The total internal resistance for the control electrode was the lowest (49700 Ω).

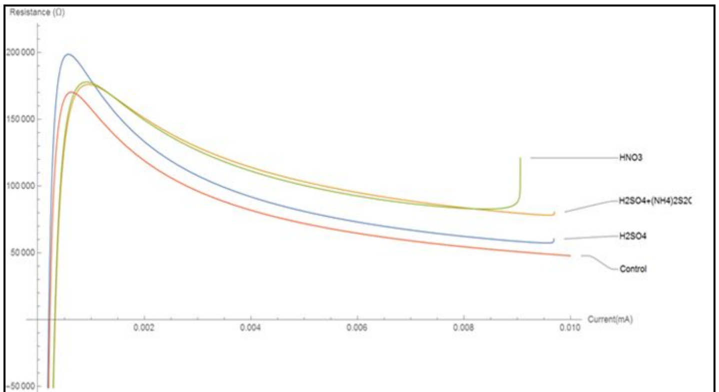

Figure 10: Graph of total internal resistance against current

Treatment	Limiting Current density (mA/cm^2)	R_{act} (Ω)	R_{conc}(Ω)	R_{ohm}(Ω)	Total (Ω)	Activation loss constants
Control	0.01359	48	48	9509	9605	a = 851.5, b = 101.81
H_2SO_4	0.00969	639	633	12881	14153	a = 904.20, b = 106.91
$H_2SO_4 + (NH_4)_2S_2O_8$	0.00970	545	546	18535	19626	a=1119.01, b = 140.64
HNO_3	0.00906	4860	4933	27966	37759	a = 1141.66, b =142.81

Table 2: Table showing distribution of internal resistance at maximum power densities, limiting current densities, and activation loss constants for microbial fuel cells with different electrodes

As seen from Table 2, modification of electrodes has increased the activation constants and the total internal resistance, with Nitric acid-treated carbon rods having the highest total internal resistance (37759)and the highest activation loss constants (a=1141.66, b=140.64). Electrodes treated with sulfuric acid were the most effective as they had relatively low a and b constants (a=904.2, b=106.9) and also a relatively high power density (0.527 mW).

5. Conclusion

Lactobacillus Acidophilus has been proven to be electrogenic, with 300 ml of *Lactobacillus Acidophilus* and ethanol generating 0.350mV using plain carbon electrode. The substrate that yields highest voltage with *Lactobacillus Acidophilus* is ethanol. The plain carbon rod had lowest resistance, but the carbon rod treated with sulfuric acid gave highest power density and as such gave highest voltage overall. The discovery of a new electrogenic bacteria has opened up many possibilities for future investigation into further optimisation of MFCs using *Lactobacillus Acidophilus*. *Lactobacillus Acidophilus* and the substrates and electrodes used in this project were also considerably cheaper than the bacteria, substrates and electrodes used in previous research into MFCs. *Lactobacillus Acidophilus* is also relatively inexpensive to culture and incubate as it is more prevalent than many bacteria used in MFCs, and is facultatively anaerobic which means it can respire aerobically and anaerobically. Hence it has become considerably more economical to invest in MFCs for large scale production of electricity as less financial capital is needed to set up an MFC.

6. Future Work

Even though a plain carbon rod had lowest resistance, it had the lowest power density. This could be due to certain chemical reactions the treated carbon rods had with the substrate or bacteria. Determining the exact reason is an interesting area for future research. Due to time constraints, datalogging of voltage over time could only be done for a maximum of 24h. In the future, datalogging could be done over 300 h to obtain more reliable and comprehensive results.

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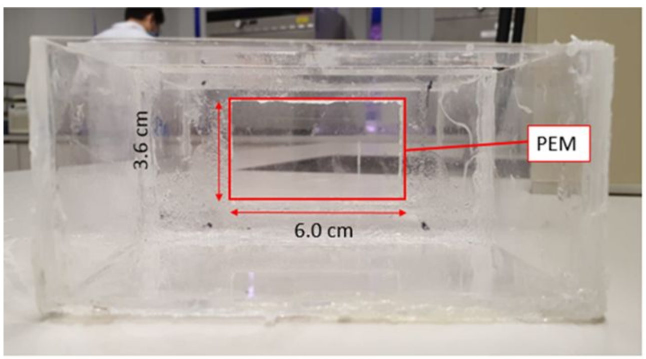
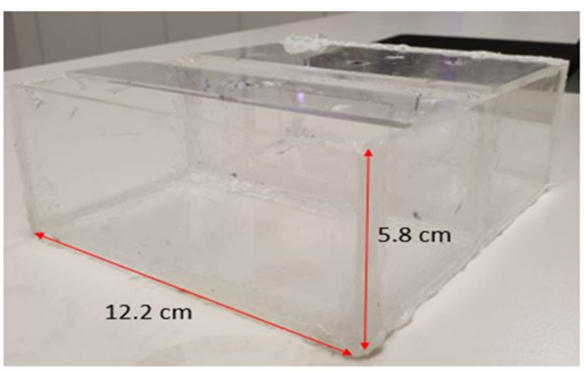
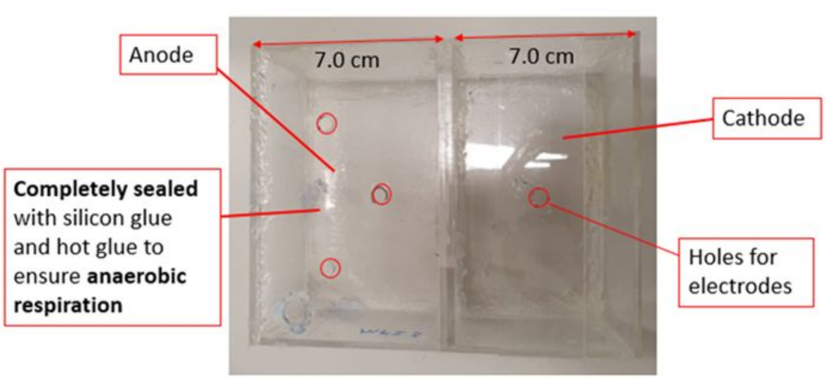
Appendices



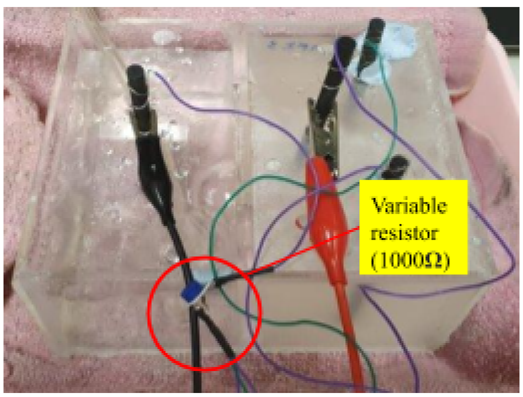
Appendix A: Photo of our setup with dimensions



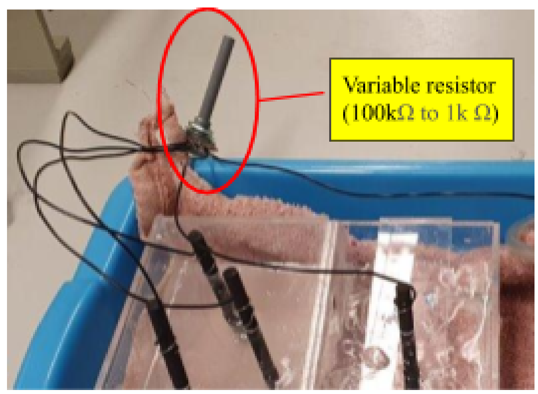
Appendix B: Photo of our setup with dimensions



Appendix D: Photo of our overnight data logging setup



Appendix E: Photo of our variable resistor



Appendix F: Photo of our variable resistor